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13. (Amended) A process for producing a transformed mammalian cell line, comprising the step of transfecting a mammalian cell with a vector according to claim 1, wherein said DNA structural sequence comprises a DNA sequence selected from the group consisting of a dihydrofolate reductase gene (DHFR), a thymidine kinase gene, a thymidylate synthetase gene, **[gene]** a DRTF1/E2F transcription factor-encoding DNA sequence, and an E2F transcription factor-encoding DNA sequence.

14. (Pending) A process for producing a transformed mammalian cell line, comprising the step of transfecting a mammalian cell with a vector according to claim 1, wherein said DNA structural sequence comprises an oncogene.

REMARKS

Applicant wishes to thank Examiner Terry McKelvey for the courtesy extended to their representative Nancy Vensko on January 22, 2001, and December 19, 2000. The Interview Summary (Form PTOL-413) summarizes the discussions held at the personal interviews. The present *Second Supplemental Amendment* includes the substance of the Examiner Interviews.

By this *Second Supplemental Amendment*, Applicant has canceled Claims 15-21 directed to an embodiment supplemental to original Claims 1-14 where the embodiment is a transfection vector that consists of an NLS only.

Additionally, by this *Second Supplemental Amendment*, Applicant has followed the steps as if to obtain a Certificate of Correction, because a mistake of a clerical or typographical nature or of minor character appears in Claims 12 and 13, was not the fault of the Office, and occurred in good faith, per Reissue Application Declaration dated 9/21/99, Supplemental Declaration For Reissue Patent Application dated 8/31/00, and Second Supplemental Declaration for Reissue Patent Application filed herewith.

Finally, by this *Second Supplemental Amendment*, Applicant acknowledges the allowability of Claims 1-14, now that the objections to objected-to Claims 12 and 13 are obviated. In the personal interviews, the claims were discussed. Our IDS identified Woo et al. (USP 5,994,109).

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Discussi n of W et al. (USP 5,994,109)

We begin with a discussion of "hinge region" in pending claim 1, turn to a discussion of Woo et al., and conclude with a discussion of how our claim 1 reads on our Example 1 and not Woo et al.

Our patent specification cites "hinge" 5 times. It shows that a hinge region of neutral amino acids that connects a basic amino acid (BAA) domain to a nuclear localization signal (NLS) domain has the following definition.

a. Comprises a stretch of neutral small amino acids taken from the group consisting of G,A,L, and I, and
b. Functions to minimize stearic interference between the two domains.

3:15	Connects polymeric chain of basic amino acids to NLS
3:20	Comprises 6-50 amino acids taken from group consisting of G, A, L, and I
7:38	Purpose = to minimize stearic interference between the two domains
7:40	6-25 amino acids and contains a stretch of neutral small amino acids without any bulky hydrophobic or ionic side chains
9:10	Optionally connects synthetic polypeptide to a cell-type specific ligand molecule

Thus, the patent specification shows that "hinge region" in pending claim 1 has the above definition.

We turn now to a discussion of Woo et al. (USP 5,994,109). Woo describes a nucleic acid transporter system comprising (a) a "binding" molecule noncovalently bound to a DNA and covalently linked to (b) a cell-surface ligand, (c) a nuclear ligand, and (d) a lysis agent, and, optionally, a spacer. Woo's spacer is defined at col 9, ¶ 1:

Optionally connects (a) to (b)(c)(d)
Optionally hydrophilic
Optionally has the formula [(gly)i(ser)j]k where i ranges from 1 to 6, j ranges from 1 to 6, and k ranges from 3 to 20

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Woo's description shows that he used Huston's linker taken from Huston et al., Methods in Enzymology 203:46, Table I, & p.52-54 (1991) (attached in IDS):

GGGGS GGGGS GGGGS

We conclude with a discussion of how our claim 1 reads on our Example 1 and not Woo et al. Here is our Example 1:

PKKKRKV SGGGGG KKKKKKKKKKKK (SEQ ID NO:56)

The definition of hinge region in claim 1 excludes those spacers that cause stearic interference. The Huston linker of Woo et al. would cause stearic interference. The hinge region of SEQ ID NO:56 would not cause stearic interference.

This is because we have "one repeating unit" of -(Gly)4-Ser- rather than "three repeating units," and "one repeating unit" would minimize stearic interference while "three repeating units" would not achieve this purpose. I.e., we're different from Huston (which Woo did not appreciate), for Huston found that "one repeating unit" did not work for him (too strained). In contrast, "one repeating unit" works for us (it's our example).

The Huston paper shows that his purpose was to connect two arms of an antibody binding site, but our purpose is to connect a BAA domain to an NLS domain. Many NLS domains contain a stretch of basic amino acids like the NLS in SEQ ID NO:56, thus we have a different problem which is to connect two positively charged domains. Woo did not appreciate this problem because he used Huston's linker.

Huston's linker would not work for us because of the distribution of serines along the length of the linker, pursuant to Stryer,[1] which says that the side chains of serine (plus threonine, asparagine, glutamine, tyrptophan, arginine, lysine, aspartate, glutamate, tyrosine, histidine) can serve as hydrogen bond donors and acceptors, thus there would be hydrogen bonding between the cationic amino acids in either domain and the serines in the mid section of the "three rep ating units" of the Huston linker.

[1] L. Stryer, Biochemistry, 3rd Ed., Freeman & Co., NY, 1988 (p.18-20, & 28-29 attached in IDS).

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Consequently, there would be stearic interference with the domains, diminishing their ability to serve their purpose (which for the NLS domain is self-explanatory and for the BAA domain is to link electrostatically to a DNA). This is as opposed to having "one repeating unit" in which the serine and the cationic amino acids in SEQ ID NO:56 (plus threonine, asparagine, glutamine, tyrptophan, arginine, lysine, aspartate, glutamate, tyrosine, histidine) would not serve as hydrogen bond donors and acceptors to form hydrogen bonds between the cationic amino acids in either domain and the serine as is achieved in the Huston linker, due to the repulsion of like-charges between positively charged domains thus forcing the domains apart, consequently minimizing stearic interference.

Accordingly, our claim 1 reads on our Example 1 and not Woo et al., because the definition of hinge region in claim 1 excludes the Huston linker of Woo which would cause stearic interference (while it includes the hinge region of SEQ ID NO:56 which would minimize stearic interference).

CONCLUSION

In view of the foregoing, Applicant respectfully requests that this *Second Supplemental Amendment* be entered. If any points remain that can be resolved by telephone, the Examiner is invited to contact the undersigned at the below-given telephone number.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: 1/30/01

By: _____

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